

In the Specification:

Please replace the paragraph beginning at page 2, line 6, with the following rewritten paragraph:

E1 -- Briefly stated, the present invention provides immunogenic synthetic fusion polypeptides which stimulate an immune response against Group A streptococci. Within one aspect such polypeptides comprise (a) at least two immunogenic polypeptides from a Group A streptococci of at least 10 amino acids in length which are capable of stimulating an immune response against Group A streptococci, and a peptide C terminal to the immunogenic polypeptide which protects the immunogenicity of the immunogenic portion. Within preferred embodiments, the C-terminal peptide is not required to stimulate an immune response against Group A streptococci and hence, may be an inconsequential non-immunogenic peptide, or a reiterated immunogenic polypeptide. Within certain embodiments, the immunogenic polypeptide can be obtained from a wide variety of Group A streptococci (ranging from "1" to greater than "90"), including for example, Types 1, 1.1, 2, 3, 4, 5, 6, 11, 12, 13, 14, 18, 19, 22, 24, 28, 30, 48, 49, 52, 55 and 56. --

Please replace the paragraph beginning at page 2, line 19, with the following rewritten paragraph:

E2 -- Within other aspects of the present invention, vaccinating agents are provided for promoting an immune response against Group A streptococci, comprising (a) at least two immunogenic polypeptides from a Group A streptococci of at least 10 amino acids in length which are capable of stimulating a protective immune response against Group A streptococci, and (b) a peptide C terminal to the immunogenic polypeptide which protects the immunogenicity of the immunogenic portion, wherein the C-terminal peptide is not required to stimulate an immune response against Group A streptococci. As above, the polypeptide may be selected from a wide variety of Group A streptococci (ranging from "1" to greater than "90"), including for example, types 1.1, 2, 3, 4, 5, 6, 11, 12, 13, 14, 18, 19, 22, 24, 28, 30, 48, 49, 52, 55 and 56. Within certain further embodiments, the vaccinating agent may further comprise an adjuvant,

Es such as, for example, alum, Freund's adjuvant, and/or an immunomodulatory cofactor (e.g., IL-4, IL-10,  $\gamma$ -IFN, or IL-2, IL-12 or IL-15). --

Please replace the paragraph beginning at page 5, line 7, with the following rewritten paragraph:

Es -- As noted above, the present invention provides vaccinating agents suitable for preventing Group A streptococcal infections. Briefly, as described in more detail below it has been discovered that, in order to optimize the immunogenicity of all aspects of a multivalent vaccine. Within one aspect of the invention, immunogenic synthetic fusion polypeptides which stimulate an immune response against Group A streptococci are provided. Such polypeptides generally comprise (a) at least two immunogenic polypeptides from a Group A streptococci of at least 10 amino acids in length which are capable of stimulating an immune response against Group A streptococci, and (b) a peptide C terminal to the immunogenic polypeptide which protects the immunogenicity of the immunogenic portion, wherein the C-terminal peptide is not required to stimulate an immune response against Group A streptococci. Particularly preferred protective peptides are generally at least ten amino acids in length, and may be 30 amino acids or longer. --

Please replace the paragraph beginning at page 18, line 28, with the following rewritten paragraph:

Es -- Opsonic M protein antibodies correlate with protection against infection with the same serotype of group A streptococci (Lancefield, R.C., "Current knowledge of the type specific M antigens of group A streptococci," *J. Immunol.* 89:307-313, 1962; Lancefield, R.C., "Persistence of type-specific antibodies in man following infection with group A streptococci," *J. Exp. Med.* 110:271-282, 1959). Two related in vitro assays are used to detect opsonic antibodies in immune sera. The first is a screening assay that measures opsonization in mixtures of immune serum, whole, nonimmune human blood and the test organism (Beachey *et al.*, "Purification and properties of M protein extracted from group A streptococci with pepsin: Covalent structure of the amino terminal region of the type 24 M antigen," *J. Exp. Med.* 145:1469-1483, 1977). 0.1 ml of test serum is added to a standard number of bacteria and incubated for 15 minutes at room

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temperature. 0.4 ml of lightly heparinized human blood is added and the entire mixture is rotated end-over-end at 37°C for 45 minutes. At the end of the rotation, smears are prepared on microscope slides that are air-dried and stained with Wright's stain. "Percent opsonization" is quantitated by counting the percentage of polymorphonuclear leukocytes that have ingested or are associated with bacteria. An interpretable assay must have a preimmune control value that is 10% opsonization or less. --

Please replace the paragraph beginning at page 19, line 17, with the following rewritten paragraph:

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-- Confirmation of the presence of opsonic antibodies is obtained by indirect bactericidal antibody assays according to the original description by Lancefield (Lancefield, R.C., "Current knowledge of the type specific M antigens of group A streptococci," *J. Immunol.* 89:307-313, 1962). This assay is performed using test mixtures as described above except that fewer bacteria are added and the rotation is allowed to proceed for 3 hours. At the end of the rotation, pour plates are made in sheep blood agar and bacteria surviving are quantitated after overnight growth at 37°C. Percent killing in the presence of immune serum is calculated by comparing to the growth in nonimmune serum. --

Please replace the paragraph beginning at page 20, line 4, with the following rewritten paragraph:

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-- Protective efficacy of M protein vaccines is determined by either indirect or direct (passive or active immunization) mouse protection tests. Indirect tests are performed by giving mice 1 ml of immune or preimmune serum via the intraperitoneal (i.p.) route 24 hours prior to challenge infections with the test organism given i.p. (Beachey *et al.*, "Human immune response to immunization with a structurally defined polypeptide fragment of streptococcal M protein," *J. Exp. Med.* 150:862-877, 1979). For each test organism, groups of 25 mice receive either preimmune or immune serum. The animals are then divided into 5 groups of 5 mice each and 10-fold increasing challenge doses of virulent streptococci are given to each subgroup. After 7 days of observation, the 50% lethal dose (LD<sub>50</sub>) is calculated for each serotype tested. --

Please replace the paragraph beginning at page 20, line 22, with the following rewritten paragraph:

E7 -- In order to show directly the protective efficacy of opsonic antibodies evoked by the hexavalent vaccine, mice were immunized with the vaccine adsorbed to ALUM and then challenged with two of the serotypes represented in the vaccine. Female outbred white Swiss mice were immunized via the i.m. route in the hind leg according to the following schedule: time 0, 25µg; 3 weeks, 25 µg; 6 weeks, 50µg; and 13 weeks, 50µg. Challenge experiments were performed on the 20 immunized mice and 20 control, unimmunized mice (Table 1). The challenge strains were types 24 and 19, with the reasoning that the M24 peptide is the largest fragment in the hexavalent protein and is reiterated and the M19 fragment is one of two that are only 35 amino acids long. These two fragments should reflect the range of protective immunogenicity of the hexavalent protein. Intraperitoneal challenge of mice with virulent streptococci is the most stringent laboratory assay for opsonic antibodies. --

Please replace the paragraph beginning at page 21, line 23, with the following rewritten paragraph:

E8 -- To assure that none of the M protein vaccines evokes tissue-crossreactive antibodies, indirect immunofluorescence assays are performed using frozen sections of human heart, kidney, and brain (Dale, J.B. and Beachey E.H., "Protective antigenic determinant of streptococcal M protein shared with sarcolemmal membrane protein of human heart," *J. Exp. Med.* 156:1165-1176, 1982). Thin sections of tissue obtained at autopsy (4µm) are prepared on microscope slides and stored in a sealed box at -70°C until use. Test serum is diluted 1:5 in PBS and dropped onto the tissue section. Control slides are made with preimmune serum and PBS. The slides are incubated at ambient temperature for 30 minutes and then washed three times in PBS in a slide holder. Fluorescein-labeled goat anti-IgG/IgM/IgA is diluted 1:40 in PBS and dropped onto the slides which are again washed, dried, and mounted with 1% Gelvetol and a coverslip. Fluorescence is detected using a Zeiss Axiophot microscope equipped with a xenon light source. Immunofluorescence is recorded using a scale of 0-4+, with 0 being no fluorescence and 4+ being that obtained with a standard, positive antiserum raised in rabbits